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DANN, DORFMAN, HERRELL & SKILLMAN
1601 MARKET STREET
SUITE 2400
PHILADELPHIA, PA 19103-2307

EXAMINER	
LEFFERS JR, GERALD G	
ART UNIT	**PAPER NUMBER**
1636	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/980,913	ARENAS ET AL.	
	Examiner	Art Unit	
	Gerald G Leffers Jr., PhD	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 January 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-15,19-21,23-43,48-54 and 58-61* is/are pending in the application.

4a) Of the above claim(s) *23-28,48-54 and 58* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-15,19-21,29-43 and 59-61* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *8/4/2003, 1/20/04*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Receipt is also acknowledged of an amendment, filed 1/20/2004, in which several claims were amended (claims 1, 10, 32-33) and in which new claims 59-61 were added. Claims 1-15, 19-21, 23-43, 48-54, 58-61 are pending in the instant application.

Receipt is also acknowledged of a petition, filed 1/09/2004, concerning the restriction requirement made on 3/19/2003. A decision on the petition was mailed to applicants by the Office on 3/3/2004. As per the summary on page 3 of the Decision, claims 1-15, 19-21, 29-43 and 59-61 are under consideration in the instant application. Claims 23-28, 48-54 and 58 remain withdrawn from consideration as being directed to non-elected inventions.

Any rejection of record in the office action mailed 7/16/2003 not addressed herein is withdrawn. This action is not final as there are new grounds of rejection presented herein that were not necessitated by applicants' amendment of the claims in the response filed 1/20/2004.

Information Disclosure Statements

Receipt is acknowledged of information disclosure statements filed 1/20/2004 and 8/4/2003. The signed and initialed PTO Form 1449's corresponding to each IDS have been mailed along with this action.

Claim Objections

Claim 2 is objected to because of the following informalities: the claim recites an acronym that is not spelled-out in its first use in the claims (i.e. FGF8). It would be remedial to amend the claim language to define the acronym in claim 2 so that it is clearly understood what

factor is referred to by the term without need to look up the term in the specification.

Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-15, 19-21, 29-31, 37-43 and 59-61 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. **This is a new rejection.**

Several of the rejected claims are directed to a method of inducing a dopaminergic cell fate on a neuronal stem cell or progenitor cell comprising expressing Nurr1 above basal levels within the precurser cell and contacting the precurser cell with one or more factors obtainable from a Type 1 astrocyte of the ventral mesencephalon (e.g. claims 1-15, 19-21). Some of the rejected claims are directed to methods of screening for receptors for the factor or factors that are obtainable from and/or secreted from the Type I astrocytes (e.g. claims 29-31, 37-43). In either case, the claims are not limited to situations where the factor(s) are provided in coculture with the Type 1 astrocytes. The rejected claims also embrace embodiments where the factor or factors are delivered apart from the astrocytes in some form as relatively isolated factors (e.g. in *in vivo* methods of inducing the dopaminergic cell fate, pharmaceutical compositions comprising

the factor or factors, methods of identifying the putative receptors for the factors where the factors are provided separately from the astrocytes, etc.). Thus, a critical element of the claimed methods is the factor(s) in isolation apart from cocultures of the Type 1 astrocytes of the ventral mesencephalon and the neuronal stem or progenitor cells.

The instant specification demonstrates an enhanced direction of neuronal precursor cells to a dopaminergic cell fate when the neuronal precursors express greater than basal levels of Nurr1 and are cocultured with Type 1 astrocytes obtained from ventral mesencephalon. Separation experiments with cocultured neuronal precursors and Type 1 astrocytes (separated by a microporous membrane) indicate that at least one factor is secreted from the astrocytes that can mediate dopaminergic development for neuronal stem cells or progenitor cells that overexpress Nurr1. However, the specification does not provide any structural characteristics for the factor or factors that mediate the observed dopaminergic development effects beyond the observation that at least one such factor appears to be secretable. In fact, attempts to provide the secreted factor or factors via cultured media obtained from cultures of Type 1 astrocytes or with membrane fragments of Type 1 astrocytes were unsuccessful. The specification concludes that the secreted factor(s) is highly labile (e.g. page 38, lines 12-28 of the instant specification). Thus, it cannot be concluded that applicants had in their possession the relatively isolated factor or factors required to practice the claimed invention in the broadly recited genus of methods embraced by the rejected claims. Nor is there any basis at all in the instant specification for the skilled artisan to envision any specific factor or combination of factors obtainable from Type 1 astrocytes that will satisfy the functional limitations of the claims. Therefore, there is no basis provided by the instant specification for the skilled artisan to envision even one embodiment of

the claimed invention where the factor or factors are not provided by the astrocytes while in coculture with the neuronal stem cells or neuronal progenitor cells.

The prior art does not offset the deficiencies of the instant specification with regard to describing factors obtainable from Type 1 astrocytes that are capable of inducing dopaminergic cell fate on neuronal precursor cells expressing Nurr1. In fact, this concept appears to be novel in the art.

Given that the claims encompass a range of methods where the critical developmental factor or combination of factors is provided apart from cultured Type 1 astrocytes, and that the claims encompass a potentially broad genus of such dopaminergic-inducing factors or combination of factors, it is necessary for applicants or the prior art to provide some description of the structural/functional characteristics of such factors in order to demonstrate possession of the broadly claimed genus of methods using such factors. For the reasons outlined above, there was no basis provided by the specification and prior art at the time of filing for the skilled artisan to envision even one such factor that would meet the functional requirements of the claimed methods, much less the broadly claimed genus of such factors or combinations of factors. Therefore, the skilled artisan would reasonably have concluded applicants were not in possession of the claimed methods.

It is noted that claims 4-5 & 59-61 do not explicitly recite that the factor or factors are provided in the presence of the Type 1 astrocytes obtained from the ventral mesencephalon.

Response to Arguments

Applicant's arguments filed 1/20/2004 in response to similar grounds of rejection made in the previous office action have been fully considered but they are not persuasive. The response

essentially argues: 1) applicants have actually reduced to practice methods where a factor or factors secreted from Type 1 astrocytes obtained from the ventral mesencephalon induced neuronal stem and/or progenitor cells to a dopaminergic cell fate, 2) the claims have been amended to recite that the factor(s) is secretable, 3) the specification provides the source of the factor(s) and the means of obtaining such factors, and 4) the specification describes sufficient identifying characteristics (i.e. secretion and induction of dopaminergic induction for neuronal progenitor cell types) to convey to the skilled artisan possession of the claimed invention.

The examiner agrees that applicants have reduced to practice an *in vitro* coculturing methodology where a factor(s) secreted from Type I astrocytes obtained from the ventral mesencephalon induces a dopaminergic cell fate on neuronal stem or progenitor cells. As indicated above, applicants have provided sufficient description for embodiments where the factor or factors is provided in the presence of the Type I astrocytes *in vitro* to convey possession of such coculturing embodiments to the skilled artisan at the time of filing. Applicants have not shown, however, that they have possession of embodiments where the factor or factors are provided in a formulation apart from in the presence of the Type I astrocytes (e.g. membrane extracts, culture media from coculture experiments). Applicants' own data inform the skilled artisan that they were unable to obtain the factor or factors in a formulation that did not require the presence of the Type I astrocytes. Thus, applicants have <u>not</u> shown the means for obtaining the factor or factors responsible for the observed inductive effect on neuronal stem or progenitor cells. In any case, arguments directed to means of obtaining the factor, or combination of factors, are better suited to a rejection on the grounds of a lack of enablement and are not

applicable against the instant grounds of rejection for a lack of written description of the broadly claimed genus of methods utilizing such factors.

Moreover, the assertion that the fact that at least one of the factors that mediates the observed induction of dopaminergic cell fate on the neuronal stem or progenitor cells is secretable emphatically does not provide a framework for the skilled artisan to reliably envision which of the many factors, or combinations of factors, that are secretable by Type 1 astrocytes are responsible for the observed functional effects on neuronal precursor cells. Could applicants, based upon the specification as filed and the state of the art at the time of filing, accurately predict which of the many factors, or combination of factors, secretable from Type I astrocytes will necessarily meet the functional limitations of the claims? Applicants did not do so at the time of filing, and based upon the instant specification and prior art, the skilled artisan would not have been able to reliably envision even one embodiment of the claimed methods where the factors are not provided in the presence of the Type I astrocytes obtained from the ventral mesencephalon. Therefore, the skilled artisan would reasonably have concluded at the time of filing that applicants were not in possession of the broadly claimed genus of such methods requiring the relative isolation of the factor or combination of factors.

Claims 29-43 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. **These are new grounds of rejection.**

Enablement is considered in view of the Wands factors (MPEP 2164.01(A)). These include: nature of the invention, breadth of the claims, guidance of the specification, the existence of working examples, state of the art, predictability of the art and the amount of experimentation necessary. All of the Wands factors have been considered with regard to the instant claims, with the most relevant factors discussed below.

Nature of the invention/Breadth of the claims: Claims 29-31 and 37-43 are directed to methods of screening for a receptor or receptors for a factor or factors that, either alone or in combination, induce a dopaminergic fate in a neural stem or progenitor cell expressing Nurr1 above basal levels. As such the claims encompass embodiments where the factors are actually obtained in some form or identified. Claims 31-36 are directed to methods of screening for factors that, either alone or in combination, induce a dopaminergic fate in neural stem or progenitor cells expressing Nurr1 above basal levels. Claims 32-33 recite the limitation that molecules obtained from Type 1 astrocytes are contacted with neuronal stem cells or progenitor cells in such a manner that allows "binding" of the astrocyte-derived molecules and the stem and/or progenitor cells. The nature of the invention is complex in that it involves the induction of a particular cell fate on precursor cells that are capable of developing along several different paths to different cell types. Such developmental processes are complex, typically involving the interaction of any number of developmental factors in precise temporal/spatial expression patterns *in vivo*. The instant claims involve identification of a factor or factors that can mediate the determination of the cell fate of precurser neuronal cells to a dopaminergic state (e.g. Th+, neuronal morphology, secretion of dopamine, etc.). For example, it is unclear how one can practice the recited methods to conclusively demonstrate that a particular protein or protein

complex is a "receptor" for one or more of the putative factors (i.e. determining that the putative receptor "binds" with the factor or factors) without being able to provide the factor or factors in an identifiable form (e.g. where the factor(s) is labeled in some fashion to distinguish it from other molecules secreted from Type 1 astrocytes). Likewise, at least in some embodiments, the invention requires some knowledge of the identity of receptor(s) for the factor or factors that are involved in the induction of dopaminergic cell fate for neural stem or progenitor cells overexpressing Nurr1.

Guidance of the specification/The existence of working examples: The instant specification demonstrates an enhanced direction of neuronal precursor cells to a dopaminergic cell fate when the neuronal precursors express greater than basal levels of Nurr1 and are cocultured with Type 1 astrocytes obtained from ventral mesencephalon. Separation experiments with cocultured neuronal precursors and Type 1 astrocytes (separated by a microporous membrane) indicate that at least one factor is secreted from the astrocytes that can mediate dopaminergic development for neuronal stem cells or progenitor cells that overexpress Nurr1. However, the specification does not teach any structural characteristics for the factor or factors that mediate the observed dopaminergic development effects beyond the observation that at least one such factor appears to be secretable. In fact, attempts to provide the secreted factor or factors via cultured media obtained from cultures of Type 1 astrocytes or with membrane fragments of Type 1 astrocytes were unsuccessful. The specification concludes that the secreted factor(s) is highly labile (e.g. page 38, lines 12-28 of the instant specification). Thus, no factor or combination of factors are identified by the instant specification that mediate the direction of neuronal stem or progenitor cells to the dopaminergic cell fate. Nor does the specification clarify

whether it is a single factor, multiple different factors or a specific combination of different factors that can mediate the complex development of dopaminergic cells from neuronal stem or progenitor cells overexpressing Nurr1. Thus, applicants' own data demonstrate the unpredictability of attempting to identify the factor or factors secreted from Type 1 astrocytes in coculture that mediate the observed effects on neuronal precursor cells, or the receptors that mediate such effects. With regard to the identification of receptors for the factor or factors responsible for the observed induction of dopaminergic cell fate, the specification provides no significant guidance as to how one would definitively identify a putative "receptor" as being such without knowing the identity of the factor or factors, or without being able to provide the factor or factors in a manner that distinguishes the factor or factors from the background of molecules secreted by Type 1 astrocytes (e.g. labeling of the factor or factors).

State of the art/Predictability of the art: The demonstration that a secretable factor, factors or particular combination of factors secreted from Type 1 astrocytes obtained from the ventral mesencephalon can mediate dopaminergic cell fate for cocultured neuronal stem or progenitor cells appears to be novel in the art. The prior art, therefore, does not appear to provide any significant guidance to the skilled artisan to practicing the recited methods in a predictable manner at the time of filing.

The amount of experimentation necessary: Given the combination of factors outlined above, particularly with regard to applicants' own inability to identify or further characterize the factor or factors responsible for the observed induction of a dopaminergic cell fate for neuronal stem or progenitor cells in cocultures of such neuronal precursors and Type 1 astrocytes, it would

have required undue, unpredictable experimentation to identify such factors and/or their receptors using the recited methods.

Claims 1-3, 6-15, 41-43 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for methods of inducing a dopaminergic neuronal fate in a neuronal stem cell or neuronal progenitor cell where the neural precursor cell expresses Nurr1 above basal levels and where the neural precursor cell is contacted with one or more factors secreted from a Type 1 astrocyte of the ventral mesencephalon while in *in vitro* coculture with the Type 1 astrocyte, does not reasonably provide enablement for any method of treatment, including the following: 1) embodiments where the induction of dopaminergic cell fate is induced *in vivo* (e.g. *in situ* within the brain of an individual), 2) where induced dopaminergic cells are provided to a subject in *ex vivo* methods of treatment, or 3) where the factor or factors responsible for the induction of dopaminergic cell fate are used as part of a pharmaceutical formulation for treatment of subject in need thereof. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims. **These are new grounds of rejection.**

The following factors have been considered in formulating this rejection (*In re Wands*, 858F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988): the breadth of the claims, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability or unpredictability of the art, the amount of direction or guidance presented, the presence or

absence of working examples of the invention and the quantity of experimentation necessary. All of the factors have been considered, with the most relevant presented below.

Nature of the invention/Breadth of the claims: All of the claims read on the treatment of individuals suffering from neurological disorders, including human subjects (e.g. Parkinson's disease). Several of the rejected claims are directed to *in vivo* embodiments of methods of inducing a dopaminergic neuronal cell fate for neural stem cells or neural progenitor cells where the neural stem cell or progenitor cell expresses Nurr1 above basal levels. The specification teaches the invention includes embodiments where the Nurr1 overexpressing cells are induced to overexpress Nurr1 *in vivo* (e.g. by recombinant means and/or treatment with factors known to induce Nurr1 overexpression such as FGF8) and where the induced cells are contacted with one or more factors that mediate the dopaminergic cell fate that are secreted by Type 1 astrocytes obtained from the ventral mesencephalon (e.g. pages 7-8, bridging paragraph; page 8, lines 26-37; page 4, lines 8-14). The only disclosed utility for such embodiments is the treatment of an individual suffering from some sort of neurological disorder (e.g. exemplified by Parkinson's disease or PD). As the specification explicitly contemplates recombinant means for inducing the dopaminergic cell fate on the neuronal precursor cells (e.g. recombinant overexpression of Nurr1 in neuronal stem or progenitor cells *in vivo*), many of the claims read on *in vitro* gene therapy.

Several of the claims read explicitly on *ex vivo* methods of therapy involving the transplantation of dopaminergic cells induced *in vitro*, including embodiments where the induction of Nurr1 overexpression is recombinant in nature (i.e. *ex vivo* gene therapy). Alternatively, several of the claims read on the direct administration of a factor, or combination of factors, secreted from Type 1 astrocytes in *in vitro* coculture that are capable of inducing a

dopaminergic cell fate on neuronal stem or progenitor cells. Finally, several of the broader claims read on combinations of the above, including co-administration of the factor(s) with dopaminergic cells produced *in vitro* to the brain of an individual.

Thus, the claims all read on some sort of treatment of a neurological disorder of the brain featuring the delivery of Nurr1-induced dopaminergic cells to the appropriate portion of the brain in a therapeutic manner, whether induction of such a cell fate on neuronal progenitor cells *in situ* and/or by providing such dopaminergic cells *ex vivo*. Therefore, the methods claimed are exceedingly complex, involving the manipulation of cell fate in a progenitor population of cells, including embodiments where the manipulation is via recombinant techniques, for therapeutic effect in the brains of individuals, including humans, suffering from neurological disorders such as Parkinson's disease.

State of the Art/Predictability of the Art: The relative skill required in the art of treating neurological disorders of the brain is very high. This high level of skill required to practice the claimed methods is exacerbated in the embodiments that feature recombinant methodologies for the overexpression of Nurr1 in the neuronal stem or progenitor cells, whether *in situ* within the brain or *ex vivo*, where a whole additional level of technical difficulties must be overcome (e.g. regulating overexpression of Nurr1 at appropriate levels, delivery of nucleic acids to the appropriate host cell population, delivery of sufficient number of dopaminergic cells to the appropriate place in the brain, long-term maintenance of transplanted cells in the brain to produce a therapeutic effect, etc.).

An analysis of the prior art as of the effective filing date of the present application shows the complete lack of documented success for any treatment based on gene therapy. In a review

on the current status of gene therapy, both Verma et al (Nature (1997) 389:239-242) and Palù et al (J. Biotechnol. (1999) 68: 1-13) state that despite hundreds of clinical trials underway, no successful outcome has been achieved (e.g. Verma et al, p. 239, 1st paragraph; Palù et al, p. 1, Abstract). The continued, major obstacles to successful gene therapy are gene delivery and sustained expression of the gene. Regarding non-viral methods for gene delivery, Verma et al indicates that most approaches suffer from poor efficiency and transient expression of the gene (p. 239, col. 3, 2nd paragraph). Likewise, Luo et al (Nature Biotechnology (2000) 18:33-37) indicates that non-viral synthetic delivery systems are very inefficient. See p. 33, Abstract and col. 1, 1st and 2nd paragraphs. While all three references indicate the promise of gene therapy, it is still a technique of the future and advancements in our understanding of the basics of gene delivery and expression must be made before gene therapy becomes a useful technique (e.g. Verma et al, p. 242, col. 2-3; Palù et al, pp. 10-11; Luo et al , p. 33, col. 1, 1st paragraph).

With regard to treatment of neurological disorders, Hsich et al teach in a post-filing review of the field (Human Gene Therapy, Vol. 13, pages 579-604, March 2002; see the entire review) that gene therapy treatment of such disorders must take into to account several factors, including: the inherent toxicity and immunogenicity of typical gene therapy vectors and accompanying damage to neurons due to inflammatory responses and edema, induction of self-antigen responses, injection injuries (e.g. hemorrhage, infection and gliosis-induced formation of epileptic foci in the brain), reluctance to carry out repeated neurosurgical procedures and the resulting need for long-term expression of transgenes *in vivo*, etc. (e.g. page 579, introductory paragraph). Hsich et al teach that although even low levels of expression of a transgene may be therapeutic, unintended consequences can readily occur if overexpression is induced in

nontargeted cells and/or overexpression in the right cell has additional effects (e.g. overexpression of nerve growth factor in the brain leading to apoptosis; formation of intracellular inclusions or increased sensitivity to dopamine toxicity in transgenic animals overexpressing α-synuclein; see page 583, first paragraph). Hsich et al teach that the use of cell vehicles, particularly neuroprogenitive cells obtained from the same subject that receives the transplanted cells (e.g. autologous ex vivo treatment) offer a potentially powerful tool that may help overcome some of the difficult obstacles in CNS gene therapy and offer *hope* of replacing damaged neurons in treating neurodegenerative diseases (e.g. pages 586-587, bridging paragraph; examiner's emphasis added). However, Hsich et al teach that several factors will need to be addressed with regard to the treatment of neurodegenerative diseases such as Parkinson's disease. These include identification of patients early in the progression of the disease before a substantial number of neurons are irreversibly lost or damaged, careful assessment of potential toxicity caused by the vector and transplanted cells or by enhanced synthesis of dopa/dopamine, and of abnormal sprouting or apoptosis caused by growth factors. Hsich et al further teach that, because the basis of intrinsic toxicity underlying Parkinson's disease in humans is not addressed in the experimental animal models used for gene therapy, the data obtained in such animal model systems may not prove predictive of effects in human patients (e.g. page 594, column 2, 2nd full paragraph).

Thus, for the reasons outlined above, the art encompassed by the claimed invention (i.e. both recombinant methods of *in vivo* or *ex vivo* gene therapy) is highly complex and unpredictable. Indeed, the recent tragic and unexpected death of a participant in a gene therapy clinical trial clearly illustrates the unpredictable nature of gene therapy (e.g. Fox, ASM

News, Feb. 2000, 66 (2): 1-3; Hsich et al at page 580, column 1, 2nd paragraph). The skilled artisan at the time the present invention was made recognized the difficulty of achieving properly regulated heterologous gene expression to induce any therapeutic effect along with the additional difficulties associated with the different gene therapy methodologies.

Many of these considerations would necessarily be present for non-recombinant methods encompassed by the rejected claims as well. For example, in embodiments where the induction of Nurr1 overexpression is induced *in situ*, growth factors such as FGF8 would have to be administered in some fashion, which may well cause unforeseen pleitropic effects similar to the experiments described by Hsich et al. Alternatively, in embodiments where cells induced by FGF8 induction *in vitro* are provided *ex vivo*, there remain issues associated with obtaining the neuronal precursors from the subject for autologous transplantation, issues associated with transplantation of heterologous dopaminergic cells in cases where the precursors are obtained from another source and issues with regard to the implantation of cells into the brain tissue. Moreover, for Parkinson's disease, the issue with regard to the inability to necessarily extrapolate the effects seen in the animal model systems to those in humans in a predictable manner would also be present in embodiments of the claimed invention that do not feature the recombinant expression of a transgene (e.g. Nurr1 and/or FGF8). Therefore, the practice of the embodiments that do not feature *in vivo* or *ex vivo* gene therapy would also have been unpredictable at the time of filing with regard to achieving a therapeutic effect in humans.

Teachings of the specification/Working Examples: The instant specification demonstrates an enhanced direction of neuronal precursor cells to a dopaminergic cell fate when the neuronal precursors express greater than basal levels of Nurr1 and are cocultured *in vitro*

with Type 1 astrocytes obtained from the ventral mesencephalon of the brain. Separation experiments with cocultured neuronal precursors and Type 1 astrocytes (separated by a microporous membrane) indicate that at least one factor is secreted from the astrocytes that can mediate dopaminergic development for neuronal stem cells or progenitor cells that overexpress Nurr1. However, the specification does not teach any structural characteristics for the factor or factors that mediate the observed dopaminergic development effects beyond the observation that at least one such factor appears to be secretable. In fact, attempts to provide the secreted factor or factors via cultured media obtained from cultures of Type 1 astrocytes or with membrane fragments of Type 1 astrocytes were unsuccessful. The specification concludes that the secreted factor(s) is highly labile (e.g. page 38, lines 12-28 of the instant specification). Thus, no factor or combination of factors are identified by the instant specification that mediate the direction of neuronal stem or progenitor cells to the dopaminergic cell fate. Nor does the specification clarify whether it is a single factor, multiple different factors or a specific combination of different factors that can mediate the complex development of dopaminergic cells from neuronal stem or progenitor cells. Thus, applicants' own data demonstrate the unpredictability of attempting to identify the factor or factors that mediate the observed effects on neuronal precursor cells, or the receptors that mediate such effects.

The specification does teach that additional known factors can enhance the apparent dopaminergic-induction effect seen for cocultures of neuronal precursors and Type 1 astrocytes (e.g. EGF, bFGP and SR112370 as measured by induction of Th+ expression; e.g. pages 34-35 of the instant specification). The specification further teaches that an additional factor in the capability of the Nurr1-overexpressing precursor cells to develop the dopaminergic cell fate is

their mitotic state. The specification teaches that the Nurr1 overexpressing cells should preferably be in a mitotic state when brought into the secretable factor(s) obtained from Type 1 astrocytes of the ventral mesencephalon (e.g. pages 36-37, bridging paragraphs). The specification teaches that the factor(s) responsible for the induction of the dopaminergic cell fate for Nurr1 overexpressing neuronal precursor cells was only obtainable from Type 1 astrocytes of the ventral mesencephalon and that Type 1 astrocytes obtained from other regions of the brain were unable to induce the dopaminergic fate for neuronal precursor cells (pages 38-39, bridging paragraphs). The specification speculates that overexpression of Nurr1 in the neuronal stem cells or progenitor cells may confer on the cells the competence to respond to specific factors, including those obtained from the ventral mesencephalic astrocytes (e.g. page 40, lines 21-25). The specification teaches that by convention the ability to release dopamine in response to membrane depolarization is the vital criterion for designation of neurochemical phenotype as dopaminergic. Applicants demonstrate a correlation between the overexpression of Nurr1 in coculture experiments and dopamine release, particularly when additional factors are added to the cocultured cells (e.g. SR11237 and bFGF; see Figure 4; page 41 of the instant specification).

Finally, the stability of induced dopaminergic neurons was assessed both *in vitro* and *in vivo* (e.g. pages 42-43 of the instant specification). For *in vitro* experiments, applicants conclude that although some cellular attrition was observed in the two weeks after removal from co-culture, a significant number of cells displayed a highly mature dopaminergic phenotype, including long elaborate processes, hypertrophic cell bodies and intense levels of TH-immunoreactivity. For *in vivo* assays, cocultured cells induced to dopaminergic cell fate were surgically injected into adult mouse corpus striatum and allowed to mature for 12 days in the

absence of any additional tropic factors or supportive cells (e.g. astrocytes, oligodendrocytes, or other neurons). The specification teaches that although cells were lost in this condition, a small but significant number of dopaminergic cells derived from the cocultured cells displayed a high level of differentiation and apparent integration into the host tissue. Applicants conclude that because some of the transplanted cells maintained or even increased their TH expression after removal from the presence of the Type 1-derived factors shows that, after dopaminergic induction, their phenotype is stable. However, the applicants further state "Since only a limited number of surviving TH+ cells could be detected, exogenously applied tropic factors or supporting cells are required for long-term survival." (pages 42-43, bridging paragraph). The specification then provides a single, prophetic working example for treatment of neurodegenerative disease in a mouse model (i.e. page 43).

The specification provides no working example where Nurr1 overexpression was stimulated in neuronal precursor cells *in situ* in the appropriate location for treatment of any particular condition (e.g. FGF-mediated stimulation of Nurr1 overexpression for neuronal stem cells and/or progenitor cells in the substantia nigra of an animal model for Parkinson's disease). No significant guidance is provided for such stimulation *in situ* that does not involve recombinant DNA technology (i.e. gene therapy). Nor does the instant specification teach any example that demonstrates that the factor(s) secreted by Type 1 astrocytes of the ventral mesencephalon are present at any concentration in any particular region of the brain (e.g. the substantia nigra) sufficient to stimulate any neurotropic development of Nurr1-overexpressing precursor cells to a dopaminergic cell fate, much less to such a degree that a therapeutic effect is achieved. Even for embodiments where *ex vivo* generated cells displaying a dopaminergic

phenotype are shown to have some ability to remain dopaminergic in a mouse model, no therapeutic effect of any kind is demonstrated. Applicants themselves teach that such *ex vivo* treatments are likely to require the exogenous application of an unspecified combination of tropic factors to allow for long term survival.

Amount of experimentation required: In consideration of all of the factors described above, the quantity of experimentation necessary to carry out the claimed invention was high and of an unpredictable nature as the skilled artisan could not rely on the prior art or the present specification to teach how to use the claimed methods to necessarily achieve a therapeutic effect in an individual suffering from a neurological disorder of the brain. For example, for embodiments where the factor or factors secreted from Type 1 astrocytes of the ventral mesencephalon are provided apart from the Type 1 astrocytes (e.g. as part of a formulation of such factors), one of skill in the art would first have to envision a means of providing such factor(s) in a relatively isolated form. Applicants' own data has shown that accomplishing this step is unpredictable and had not been achieved at the time of filing. For embodiments where the cells overproducing Nurr1 are generated by recombinant DNA technology (i.e. gene therapy methods), it is clear from the prior art that although such methodologies offer hope for the future, practicing such methods at the time of filing were not predictable with regard to therapeutic success. Finally, for embodiments where the dopaminergic cells of the invention are generated *in vitro* with nonrecombinant means, the skilled artisan would have had to overcome the difficulties known in the art at the time of filing for therapeutic administration of cells to the brain and determine the composition and means of delivery for the additional factors taught by applicants necessary for long-term survival of sufficient numbers of the transplanted

dopaminergic cells to achieve any therapeutic effect. In each case, the experimentation required would have been of a trial-and-error nature, at best, and would have been undue, unpredictable experimentation. Further, even assuming that the skilled artisan would have been able to obtain a therapeutic effect in an animal model system (which is not conceded here), the prior art teaches such effects are not necessarily predictive of success in treating human neurological disorders. Therefore, while the instant specification is enabling for methods of inducing a dopaminergic neuronal fate in a neuronal stem cell or neuronal progenitor cell where the neural precursor cell expresses Nurr1 above basal levels and where the neural precursor cell is contacted with one or more factors secreted from a Type 1 astrocyte of the ventral mesencephalon while in *in vitro* coculture with the Type 1 astrocyte, it does not reasonably provide enablement for any method of treatment based upon the induced dopaminergic cells.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 19-21, 29-33, 36-43 and 59-61 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. **These are new rejections.**

Claims 19 and 59 are vague and indefinite in that the metes and bounds of the phrase "in accordance with" claim 1 or claim 4 are not clear. Does the phrase "in accordance with" mean that one necessarily practices the method of the dependent claim from which claim 19 or claim 15 depends, or only that the recited cells are obtained with a similar method that achieves the same result? Upon reading the specification, it appears the cited phrase is intended to indicate

that the product is necessarily obtained by practicing the method of the claims upon which the rejected claims are dependent (i.e. the claims are intended to be product-by-process claims). It would be remedial to amend the claim language to clearly indicate which of the two possibilities is intended by the cited phrase.

Claim 29 comprises the limitation of contacting cells with "one or more factors obtainable from a Type 1 astrocyte" for which the metes and bounds are unclear. For example, it is unclear whether the factor is necessarily obtained from a Type 1 astrocyte or whether that it is merely "obtainable" from a Type 1 astrocyte. Further, it is unclear the conditions during which the factor is "obtainable" (e.g. development, following treatment with particular growth factors, passage in culture, etc.). It would be remedial to amend the claim to read "obtained from".

Claim 29 is vague and indefinite in that there is no clear nexus between the stated outcome of the method and the actual methods steps recited in the claims, making it unclear as to what is actually claimed. For example, the claims are directed to "[A] a method of screening for a receptor or receptors for the factors or factors which are obtainable from Type I astrocytes" without any recitation that the factor or factors is present in the assay. This appears to be a missing element required to practice the claimed invention as it is unclear how one could practice the claimed method and *necessarily* obtain such a receptor without using the factor or factors themselves. Moreover, it is unclear how the simple act of "comparing neural stem or progenitor cells with or without expression of Nurr-1 above basal levels within the neural stem or progenitor cells" will necessarily satisfy the claim limitation of screening for a receptor or receptors when there is no further limitation as to what properties are to be assessed during the "comparison". As written, the claim appears to be merely a recitation of a desired result without

any clear nexus between the recited steps of the method and the end result desired. It is not clear that one would necessarily achieve the stated outcome of the claims by practicing the recited steps. It would be remedial to amend the claim language to provide a clear nexus between the stated result and the methods steps such that when one practices the recited methods steps one actually accomplishes the desired result.

Claim 30 is vague and indefinite in that the metes and bounds of the phrase "...which further comprises isolating and/or purifying and/or cloning said receptor or receptors..." are unclear. First, it is unclear how one actually clones a protein (i.e. the receptor(s)). It would be remedial to amend the claim to recite that it is the gene or genes encoding the receptor(s) that is cloned. Second, it unclear the qualitative difference intended between the terms "isolating" and "purifying". The specification does not appear to delineate between the two concepts, making it unclear what degree of isolation is required to satisfy each of the limitations. It would be remedial to amend the claim language to more clearly indicate what is intended by either of the two terms.

Claims 29, 32 and 34 are vague and indefinite in that the metes and bounds of the phrase "screening for a factor or factors which, either alone or in combination, induce a dopaminergic fate in a neural stem or progenitor cell expressing Nurr1 above basal levels" are unclear. It is unclear whether the phrase means that the factor or factors is necessarily identified or obtained or whether, like in the instant application, the phrase encompasses embodiments where only the presence of such factors is shown. It would be remedial to amend the claim language to clearly indicate whether one need only show that such a factor is present, or whether one must identify or obtain the factor itself.

Claim 36 is vague and indefinite in that the metes and bounds of the phrase "...which comprises differential expression screening..." are unclear. The claim has open "comprising" language and merely recites that differential expression screening is used in some fashion without relating how such screening fits into the method scheme. Therefore, it is unclear how one would practice the method to achieve the desired result. It would be remedial to amend the claim language to clearly indicate how the differential expression screening fits into the recited methodology and contributes to the stated end result.

Claim 37 recites "wherein a factor or factors able to induce dopaminergic fate in a neural stem or progenitor cells expressing Nurr1 above basal levels is or are provided in isolated and/or purified form". It unclear the qualitative difference intended between the terms "isolated" and "purified". The specification does not appear to delineate between the two concepts, making it unclear what degree of isolation is required to satisfy each of the limitations. It would be remedial to amend the claim language to more clearly indicate what is intended by either of the two terms. The phrase is further vague and indefinite in that it is unclear as to when the factor(s) is "provided". Read one way, the term "provided" implies the factor(s) to be tested are provided in a relatively pure form. Read in the alternative, it appears that one obtains the factor or factors in relatively pure form at the end of the screening method of claim 31, upon which claim 37 depends. It would be remedial to amend the claim language to clearly indicate which of the two possibilities is intended for claim 37.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

The rejected claims are product-by-process claims directed to "a" dopaminergic neuron produced by the methods of the invention featuring contacting neuronal stem or neuronal progenitor cells with one or more factors secreted from Type 1 astrocytes obtained from the ventral mesencephalon.

Claims 19-21 and 59-61 are rejected under 35 U.S.C. 102(e) as being anticipated by Bowen et al (U.S. Patent No. 6,284,539; see the entire patent). **This rejection is maintained for reasons of record in the office action mailed 7/16/2004 and repeated below. The rejection is extended to new claims 59-61.**

Bowen et al teaches a novel method of directing cell fate for precurser cells of the central nervous system, comprising the introduction and expression of gene coding for the nuclear receptor Nurr1 to direct neuronal precursors to a dopaminergic cell fate (e.g. Abstract; Example 4, column 12). Bowen et al teach compositions comprising differentiated neuronal precursors and additional components (e.g. forskolin in Example 4). There is no evidence of record to indicate that the dopaminergic cells obtained by the methods of Bowen et al would be distinguishable by some structural/functional feature from those claimed herein. The methods of

the Bowen et al patent and the instant specification both feature the expression of Nurr1 to drive the developmental fate of the neuronal precurser cells. There is no reason or expectation that the cells obtained from the instant methods somehow change the character of the obtained dopaminergic cells as compared to those obtained by Bowen et al.

Because the Office does not have the facilities for examining and comparing the applicant's product with the products of the prior art, the burden is on the applicant to show a novel or unobvious difference between the claimed products and the products of the prior art (e.g. that the products of the prior art do not possess the same material structural and functional characteristics of the claimed product). See in re Best, 562 F.2d 1252, 195 USPQ 430 (CCPA 1977).

Response to Arguments

Applicant's arguments filed in the response of 1/20/2004 have been fully considered but they are not persuasive. The response essentially argues that the cells produced by the methods of Bowen et al are not the same as those produced by applicants' method. In support of this line of reasoning, the response argues: 1) applicants' methods produce a much higher quantity of Th+ cells than do the methods of Bowen et al (~90% versus ~1.77%), 2) such a low number of Th+ cells can be accounted for by an up-regulation in expression of TH, which does not represent an indication of a dopaminergic phenotype, 3) the properties of the Th+ cells taught by Bowen et al are different from those obtained by the instant methods (e.g. with regard to morphology and expression of MAP2).

First, it is noted that applicants' claims are directed to "A" dopaminergic neuron produced according to applicants' methods. Thus, arguments directed, for example, to the

proportion of cells expressing Th+ markers are not as effective as they would be for claims directed to a population of cells produced by applicants' methods. It is further noted that there are no explicit limitations in the claims that the cells express Th+ at certain levels or possess a particular morphology. The assertion that the expression of TH does not correlate to a dopaminergic phenotype is contradictory to applicants' own specification which teaches that TH expression is in fact a marker for dopaminergic cell fate (e.g. page 11, lines 10-17). With regard to the assertions concerning MAP2 expression, the second passage cited by applicants' response appears to indicate that at least some of the Th+ cells tested did in fact co-stain for MAP2a/b (i.e. *few* of the cells express both markers). It is also noted that applicants do not appear to present in their own specification data that would indicate the relative expression of MAP2a/b in their cells. Likewise, applicants do not present data that shows that *all* of their Th+ cells display a neuronal morphology, or a particular degree of neuronal morphology, and there is no convincing evidence that *not a single cell* of the Th+ cells taught by Bowen et al possesses at least some degree of neuronal morphology.

Claims 19-21 and 59-61 are rejected under 35 U.S.C. 102(e) as being anticipated by Weis et al (U.S. Patent No. 5,981,165 A; see the entire patent). **This is a new rejection.**

Weis et al teach a culture method for inducing the expression of tyrosine hydroxylase in neural cells where mammalian CNS neural cells are cultured in the presence of a fibroblast growth factor and at least one member selected from the group consisting of a member of the TGF-B family of growth factors, a feeder layer bed of cells and conditioned media. For example, Weis et al teach an embodiment where single, undissociated six-day-old second

passage neurospheres were labeled with BrdU and plated onto a confluent bed of striatal-derived astrocytes. Twenty-four hours post-plating, the cells were processed for dual-label indirect immunocytochemistry for tyrosine hydroxylase activity and BrdU labeling. At least some of the Th+ cells were also shown to be MAP2-immunoreactive and demonstrated other neuronal characteristics in addition to morphology (e.g. Figure 3C; column 5, lines 44-54).

Because the Office does not have the facilities for examining and comparing the applicant's product with the products of the prior art, the burden is on the applicant to show a novel or unobvious difference between the claimed products and the products of the prior art (e.g. that the dopaminergic cells obtained by Weis et al do not possess the same material structural and functional characteristics of the claimed product). See in re Best, 562 F.2d 1252, 195 USPQ 430 (CCPA 1977).

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Gerald G Leffers Jr., PhD whose telephone number is (571) 272-0772. The examiner can normally be reached on 9:30am-6:00pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Remy Yucel can be reached on (571) 272-0781. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Gerald G Leffers Jr., PhD
Primary Examiner
Art Unit 1636

ggl

GERRY LEFFERS
PRIMARY EXAMINER